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(54) Title: CREATION OF HUMAN TUMORIGENIC CELLS AND USES THEREFOR

(57) Abstract: Disclosed are tumorigenic human somatic cells produced by introducing exogenous DNA into normal (parental) human somatic cells, in which the DNA is expressed, resulting in production of tumorigenic human somatic cells; progeny of the parental cells are also tumorigenic. Methods of producing tumorigenic human somatic cells and uses for the cells are also disclosed.



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Creation of Human Tumorigenic Cells and Uses Therefor

BACKGROUND OF THE INVENTION

During malignant transformation, cancer cells acquire genetic mutations that override the normal mechanisms controlling cellular proliferation. Little is known
5 about the precise constellation of genetic changes that are required in concert to convert a normal human cell into a tumorigenic cell, and it would be very useful to have defined populations of tumorigenic human cells in order to understand the number and identity of these genetic changes, to study the biochemical consequences of these genetic changes, and to test candidate therapeutic drugs on
10 such cells.

SUMMARY OF THE INVENTION

As described herein, Applicants have succeeded in transforming normal human somatic cells into tumorigenic cells. That is, normal human somatic cells have been changed from their normal growth state, as further described below, to an
15 abnormal state in which they form tumors *in vivo*. Thus, the present invention relates to tumorigenic human cells comprising exogenous DNA whose expression and/or function in normal human somatic cells (also referred to as parental or antecedent cells) results in transformation of the normal cells into human cells that grow in an anchorage-independent manner and form tumors *in vivo*; methods of
20 producing tumorigenic human cells and uses of the tumorigenic human cells as described herein.

A tumorigenic human somatic cell of the present invention comprises exogenous DNA which, when expressed or functions in a normal cell (in a cell that cannot grow in an anchorage-independent manner or form tumors when implanted
25 into an immunocompromised mouse) results in production of a tumorigenic cell, which is a cell that grows in an anchorage-independent manner and forms a tumor(s) in an immunocompromised mouse and generates progeny that grow in an anchorage-independent manner and form tumors in immunocompromised mice. In one embodiment, the tumorigenic cells form tumors in immunocompromised mice
30 and the tumors are non-invasive and non-metastatic. Particularly preferred embodiments of the invention are tumorigenic human somatic cells comprising

exogenous DNA characteristic of (present in) malignant (cancerous) human tumors that develop in individuals.

In essence, a normal, antecedent or parental cell, which cannot grow in an anchorage-independent manner or form tumors *in vivo*, is modified by introduction of exogenous DNA, whose expression or function results in production of a tumorigenic human somatic cell which grows in an anchorage-independent manner and forms a tumor(s) *in vivo*. In the embodiments in which the exogenous DNA is expressed, it is stably expressed and may be present episomally or integrated into the genome of the cell (into genomic DNA). In one embodiment, a tumorigenic human somatic cell has incorporated into its genomic DNA exogenous DNA which, when expressed in a normal human somatic cell, transforms the normal somatic cell and its progeny into tumorigenic human cells which are a variant of the parental cell. That is, in this embodiment, a tumorigenic human somatic cell, produced by introducing exogenous DNA into a normal parental cell, has incorporated into its genomic DNA exogenous DNA whose expression results in production of a cell that, unlike the normal, parental cell, grows in an anchorage-independent manner and forms tumors in the recipient mouse. In a second embodiment, a tumorigenic human somatic cell comprises DNA that itself acts (e.g., by inactivating a tumor suppressor gene, activating a proto-oncogene resident in the cell or activating or enhancing (prolonging or increasing in amount) expression of an endogenous gene, such as a gene encoding telomerase catalytic subunit) to transform the normal cell (and progeny thereof) into tumorigenic cells. Alternatively, exogenous DNA introduced into cells can comprise DNA that is expressed in the recipient cells and DNA that activates or turns on an endogenous gene (e.g., telomerase, a proto-oncogene); together, they result in production of tumorigenic cells.

As used herein, the term oncogene includes both genes which encode oncoproteins (e.g., oncoproteins) and genes which do not encode oncoproteins but whose function in normal human cells results in or contributes to production of tumorigenic human cells. Genes that act or function to inactivate tumor suppressor genes, activate proto-oncogenes or activate or enhance expression of a gene encoding telomerase catalytic subunit are examples of the latter. Expression and/or function of such oncogenes in normal cells in which telomerase catalytic subunit is

ectopically expressed results in production of tumorigenic cells from the parental normal human somatic cell. An oncogene is, for example, an altered form of a normal cellular proto-oncogene; a viral gene that mimics a cellular oncogene; a cellular oncogene that operates to inactivate a cellular tumor-suppressor gene; a viral
5 oncogene that operates to inactivate a cellular tumor-suppressor gene or gene product; or a cellular tumor suppressor gene in normal or altered form that operates to inactivate a cellular tumor suppressor gene. As used herein, the term oncogene further includes introduced exogenous DNA that alters directly the structure of one or more resident, endogenous gene(s) within the previously normal somatic cell
10 (e.g., by homologous recombination between the introduced DNA and the resident gene(s)) and, as a result, changes the activity or function of the endogenous gene(s) (e.g., inactivation of a resident tumor suppressor gene or activation of a resident proto-oncogene). Included in the definition of oncogene is the entire gene, as well as fragments thereof which, when introduced into normal human somatic cells, alter
15 (e.g., via homologous recombination or other genetic mechanism) the structure or configuration of resident genes within normal human somatic cells. DNA encoding an oncogene and DNA comprising an oncogene are used interchangeably herein. DNA encoding an oncogene can be the nucleic acids of the oncogene itself or DNA that encodes an oncoprotein.

20 In one embodiment, the exogenous DNA introduced into normal human somatic cells to produce tumorigenic human somatic cells comprises DNA which encodes a telomerase catalytic subunit, such as human telomerase catalytic subunit, and DNA which encodes at least one oncogene; co-expression of the two types of DNA results in production of a tumorigenic human somatic cell that is a variant of
25 the normal cell into which the exogenous DNA was introduced. Alternatively, the exogenous DNA can comprise DNA that turns on (activates) or enhances expression of a resident gene, such as a telomerase gene or proto-oncogene. In one embodiment, DNA which encodes at least one oncogene is an oncogene or mutant gene present in/characteristic of a human tumor cell. The resulting variant shares
30 many of the characteristics of the normal cell, but is tumorigenic.

In another embodiment, DNA introduced into the normal human somatic cell comprises: (1) DNA encoding a telomerase catalytic subunit, such as human

telomerase catalytic subunit, (2) DNA encoding a first oncogene and (3) DNA encoding a second oncogene. The first encoded oncogene and the second encoded oncogene are two different oncogenes and each functions in a different biochemical pathway, such as a signaling pathway, in the cell. For example, DNA encoding a telomerase catalytic subunit; DNA encoding an oncogene which functions in the same signaling pathway as does the mutant *H-ras* oncogene product (DNA encoding a functional equivalent of mutant *H-ras* oncogene product) and DNA encoding an oncogene which functions in the same signaling pathways as does the SV40 large T (LT) antigen-encoded oncoprotein (DNA encoding a functional equivalent mutant of SV40 LT antigen) are introduced into a normal human somatic cell, in which they are co-expressed. An oncogene which functions in the same signaling pathway as the mutant *H-ras* oncogene can function upstream of the location/position at which the mutant *H-ras* oncogene functions, downstream of that location/position or at the same location/position in the pathway. This is also the case for an oncogene which functions in the same signaling pathway as does SV40 LT antigen oncoprotein. DNA introduced into normal human somatic cells can be, in addition to DNA encoding telomerase catalytic subunit, other combinations of two or more DNAs, each of which is a different oncogene. These combinations include, for example, mutant DNAs present in (characteristic of) malignant human tumors.

In the embodiments described herein, the resulting recipient cell (which contains the exogenous DNAs) is cultured in such a manner that it propagates. In one embodiment, expression of exogenous DNA in the recipient human somatic cell and the continued presence and expression of the two types of DNA in its progeny causes them to be tumorigenic. In one embodiment, the DNAs are integrated into genomic DNA and expressed. In a specific embodiment, DNA encoding human telomerase catalytic subunit; DNA encoding an oncogene that functions in the same signaling pathway as does mutant *H-ras* oncogene product and DNA encoding an oncogene that functions in the same signaling pathway as does SV40 LT antigen oncoprotein is introduced into a normal human somatic cell, becomes incorporated into genomic DNA of the recipient cell and is expressed. The resulting expression products (human telomerase catalytic subunit, and the two oncoproteins, such as

mutant H-*ras* oncoprotein and SV40 LT antigen oncoprotein, affect the recipient cell in such a manner that the recipient cell and its progeny grow in an anchorage-independent manner and form tumors *in vivo* (e.g., after introduction into an immunocompromised mouse).

5 DNA introduced into and expressed in the normal human somatic cell can also comprise (a) DNA encoding human telomerase catalytic subunit and (b) at least one of the following additional DNAs: DNA that inactivates or DNA that encodes a product that inactivates a tumor suppressor gene; DNA that encodes a product that activates or DNA that activates a proto-oncogene in the normal cell; and DNA that
10 encodes an oncogene product. In one embodiment, DNA of (a) and two or more of the types of DNA described in (b) are introduced into normal human somatic cells. In another embodiment, DNA introduced into a normal human somatic cell can be (a) DNA whose expression or function in the cell results in activation of an endogenous gene encoding telomerase catalytic subunit (e.g., hTERT) and (b) at
15 least one of the following additional DNAs: DNA that inactivates or DNA that encodes a product that inactivates a tumor suppressor gene in the normal cell; DNA that activates or DNA that encodes a product that activates a proto-oncogene in the normal cell; and DNA that encodes an oncogene product.

Alternatively, human telomerase catalytic subunit itself can be introduced
20 into normal human somatic cells along with at least one of the DNAs described in above.

The normal human somatic cell that is transformed by the method described herein to produce a tumorigenic human somatic cell can be any somatic cell type for which a corresponding tumorigenic cell is desired. The modified normal human
25 somatic cell is cultured under conditions appropriate for propagation/replication of the cell and expression/function of the exogenous DNA. Alternatively any oncogene or a combination of two or more oncogenes can be introduced into a normal human somatic cell with human telomerase catalytic subunit in order to transform the normal human somatic cell into a tumorigenic cell. The modified
30 normal human somatic cell is cultured under conditions appropriate for propagation/replication of the cell and expression /function of the human telomerase catalytic subunit and the exogenous DNA. The ability of a specific oncogene or

combination of oncogenes to convert a normal human somatic cell into a tumorigenic variant can be determined using known methods, such as those described herein (e.g., determining the ability of cells to grow in an anchorage-independent manner and to form tumor(s) *in vivo*). A variety of cell types, including
5 normal human kidney epithelial cells, human foreskin fibroblasts, primary human mammary epithelial cells and small airway epithelial cells have been converted into tumorigenic cells. The resulting cells grow in an anchorage independent manner and form tumors when implanted into animals; the site of injection does not alter these properties.

10 In one embodiment, tumorigenic human somatic cells are produced by introducing into normal human somatic cells exogenous DNA which comprises exogenous DNA encoding telomerase catalytic subunit (or causes activation of an endogenous subunit-encoding gene) and exogenous DNA that encodes at least one oncogene product, to produce normal human somatic cells containing the exogenous
15 DNA (also referred to as recipient cells); culturing the normal human somatic cells containing the exogenous DNA under conditions appropriate for (which result in) stable maintenance of the exogenous DNA in the cells and expression and function of the respective encoded products (telomerase catalytic subunit and oncogene product(s)), thereby resulting in alteration of the recipients. Alternatively,
20 tumorigenic human somatic cells are produced by activating or enhancing expression of at least one endogenous gene (such as an endogenous human telomerase catalytic subunit gene, a proto-oncogene) and/or inactivating at least one endogenous gene (e.g., a tumor suppressor gene) and/or introducing one or more oncogenes into normal human somatic cells. Any of these combinations can be used
25 to produce tumorigenic human somatic cells from normal human somatic cells, using the methods described herein. The recipient cells grow in an anchorage-independent manner and form tumors *in vivo* (e.g., after introduction into immunocompromised mice). Cells produced in this manner and progeny thereof are tumorigenic and can be used in the methods described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A and 1B are graphs showing data that demonstrate that *hTERT* immortalizes LT-expressing HEK and BJ cells. Figure 1A represents growth of HEK cells. Figure 1B represents growth of BJ cells. Cells lacking *hTERT* (circles) entered crisis and could no longer be passaged. Filled symbols represent cells that express oncogenic *ras*. Squares indicate cells expressing *hTERT*.

Figures 2A and 2B are graphs showing data which represent growth properties of tumorigenic cells. Figure 2A show that explanted or parental HEK cells expressing LT, *ras* and *hTERT* grew at equivalent rates in culture. HEK cells expressing LT and *hTERT* but lacking (open circles) or expressing (open squares) *ras* were compared to explanted tumor cells (filled squares). Figure 2B shows that explanted tumor cells grew at the same rate as parental HEK cells expressing LT, *ras* and *hTERT* upon re-inoculation into nude mice. Symbols are the same as in Figure 2A. Mean +/- SD for six experiments is shown.

15 DETAILED DESCRIPTION OF THE INVENTION

As described herein, for the first time, normal human somatic cells have been transformed into tumor cells by the introduction of exogenous genes; the resulting cells and their progeny exhibit characteristics indicative of tumorigenic cells. For example, the cells grow in an anchorage independent manner and form tumors *in vivo*. The normal human somatic cell which can be manipulated as described herein to become tumorigenic can be any type of normal human somatic cell for which a tumorigenic cell is desired. For example, normal human somatic cells which can be transformed by the present method to produce variants of the parental cells which are tumorigenic include cells from the central and peripheral nervous system; the ocular system; connective tissues; the cardiovascular system; vasculature including arterial, venous, and capillary systems; pulmonary; muscular system including smooth and striated muscle; hepatic tissue; intestinal system, including oral, laryngeal, the esophageal, gastric, ampullary, biliary, pancreatic, small and large intestine, rectal, anal tissues; blood forming tissues; lymphatic tissues; female and male reproductive tissues, including breast, ovarian, fallopian, cervical, vaginal, testicular, prostatic, and penile tissues; genitourinary tissues such as kidney, bladder,

ureteral tissues; and skin, including epidermis, dermis and melanocytes. Cells comprising each of these tissues, including epithelial, stromal, neural, vascular, and specialized examples of each of these types can be used.

As a result of the work described herein, a wide variety of tumorigenic
5 human somatic cells, each of which is derived from a normal cell, can now be produced, opening up avenues of investigation and assessment that previously did not exist because, until now, normal human somatic cells could not be rendered tumorigenic through introduction of a defined set of genes. For example, cells of the present invention are useful to screen for drugs that kill tumor cells or make them
10 more susceptible (vulnerable) to other drugs and thus increase the effectiveness of the other drug(s). Furthermore cells of this invention are also useful to identify genes that cause tumorigenic cells to metastasize.

Thus, the present invention encompasses tumorigenic human somatic cells produced by introducing exogenous DNA, as defined herein, into normal human
15 somatic cells (or a precursor of the tumorigenic cells), which are cultured/propagated under conditions which result in expression/function of the exogenous DNA and production of tumorigenic cells and methods of producing such tumorigenic cells. It also relates to uses for the resulting cells, such as in methods of identifying (screening for) drugs useful in killing tumorigenic cells and/or reducing
20 proliferation of tumorigenic cells, particularly drugs that selectively kill and/or selectively reduce proliferation of tumorigenic cells (compared with their effects on normal cells), diagnostic methods in which tumor-specific products are used to identify and/or quantify tumorigenic cells; methods of assessing the stage of tumor progression and/or prognosis of an individual and methods of producing reagents
25 (e.g., antibodies, nucleic acids, proteins) useful in the screening, diagnostic and/or clinical methods. It further relates to drugs identified using the tumorigenic human somatic cells and reagents produced using the tumorigenic human somatic cells.

One type of DNA introduced into the normal cells in one embodiment of the method of the present invention is DNA encoding the telomerase catalytic subunit of
30 a telomerase holoenzyme. The DNA can be genomic DNA or cDNA and can be from a wide variety of organisms in which it occurs naturally (e.g., human, mouse, pig, rat, dog, monkey), provided that, when it is expressed or functions in human

somatic cells, it produces a product which has substantially the same function as does human telomerase catalytic subunit. Alternatively, DNA encoding telomerase catalytic subunit can be produced using recombinant DNA methods or can be chemically synthesized. As used herein, the term "DNA encoding telomerase catalytic subunit" encompasses DNA obtained from or produced by any of these sources or methods. In one embodiment of the present invention, the DNA encodes the human telomerase catalytic subunit and, in a specific embodiment, is *hTERT*. (Nakamura, T.M. and Cech, T.R., *Cell*, 92:587-590 (1998), Meyerson, M. et al., *Cell*, 90,: 785-795 (1997)). Alternatively, exogenous DNA that does not itself encode telomerase catalytic subunit, but activates or enhances expression of an endogenous gene encoding the subunit is introduced into normal human somatic cells, in which it activates a "silent" endogenous gene encoding the catalytic subunit or enhances expression of the endogenous gene encoding the catalytic subunit already being expressed in the cell.

The second type of DNA introduced into the normal cells in the present method is DNA comprising at least one oncogene. The oncogene can be any oncogene which, when expressed or functional in normal human somatic cells in which telomerase catalytic subunit is ectopically expressed, results in the production of the tumorigenic cells upon culturing/propagation of the recipient normal human cell. Preferably, the oncogene(s) introduced into normal human somatic cells is an oncogene(s) characteristics (causative) of malignant human tumors. As a result, the tumorigenic cells produced contain an oncogene(s) present in naturally occurring human malignant or cancerous tumors and are useful in methods described herein such as methods of assessing the ability of a candidate drug to inactivate or inhibit the oncogene(s) or render tumor cells more vulnerable to other drugs or forms of treatment, such as radiation or laser therapy.

As used herein, the term "DNA comprising an oncogene" encompasses DNA whose expression results in production of an oncoprotein, such as an oncoprotein, and DNA which itself functions as an oncogene, such as by inactivating a resident tumor suppressor gene or by activating a resident proto-oncogene. It encompasses DNA that comprises or encodes all of the types of oncogenes described herein. The oncogene can be, for example, *H-ras* and *K-ras*, *her2-neu*, *RET*, *sis*, (PDGF) N-

myc, *L-myc*, *c-myc*, *bcl-1*, *bcl-2*, *src*, and its family of related genes, MDM2 and any oncogene found in human tumor cells. The oncogene can also be a viral oncogene, such as SV40 large T, polyoma middle T, human papillomavirus E6, E7, and the Epstein-Barr virus, and hepatitis B virus and tumor suppressor genes such as: APC, DPC4, NF-1, NF-2, p53, RB, MTS1, WT1, BRCA1, BRCA2, VHL, and PTEN. Entire oncogenes or portions thereof sufficient to result in the production of tumorigenic cells can be used. One oncogene or a combination of two or more oncogenes, such as a combination of two or more of the oncogenes listed, can be co-expressed with ectopically expressed telomerase catalytic subunit to produce tumorigenic cells.

More than one DNA encoding an oncogene can be used in the present method to produce tumorigenic cells. In the embodiments in which two or more oncogene-encoding DNAs are introduced, the DNAs encode oncogenes which function in biochemically distinct manners from one another. For example, each DNA can encode an oncogene that functions in a different biochemical (e.g., signaling) pathway. That is, for example, if two oncogene-encoding DNAs are used, each encodes or activates/enhances expression of an oncogene which functions in a distinct pathway. Any number of oncogene-encoding DNAs can be introduced into normal human somatic cells in which telomerase is ectopically expressed in order to render them tumorigenic. For example, in some instances it is desirable to introduce three, four, five, six or even more oncogene-encoding DNAs. The DNAs can each function in/affect a different signaling pathway or more than one can function in/affect a common pathway.

The two types of DNA introduced in the present method can be obtained from a variety of sources. For example, they can be cloned DNA, DNA obtained from a source in which they occur in nature or DNA produced by synthetic or recombinant DNA methods. They can be cDNA or genomic DNA. They can be introduced into the normal human somatic cells by a variety of techniques, such as by means of an appropriate vector (e.g., a retrovirus, such as an amphotropic retrovirus; an adenovirus vector; lentivirus); calcium phosphate-mediated transfection; lipofection; microinjection; microparticle bombardment; RNA transfection; naked DNA injection or electroporation). The resulting normal human

somatic cells contain exogenous DNA encoding telomerase catalytic subunit and exogenous DNA comprising at least one oncogene; expression and/or function of the DNAs results in production of tumorigenic human somatic cells from the parental normal cells. Progeny of such cells are tumorigenic.

5 Thus, one embodiment of the method of the present invention is a method of producing tumorigenic human somatic cells from normal human somatic cells by introducing into the normal (parental) cells exogenous DNA comprising DNA encoding a telomerase catalytic subunit and DNA encoding at least one oncogene, to produce normal (recipient) human somatic cells containing the exogenous DNA and
10 culturing the recipient cells under conditions appropriate for (under conditions which result in) stable maintenance of the exogenous DNA in the recipient cells, expression of the exogenous DNA to produce the encoded products (telomerase catalytic subunit, oncoprotein(s)) and function of the encoded products (e.g., function of the oncoprotein(s) in signaling pathway(s)) in the recipient cells,
15 resulting in transformation of normal cells into tumorigenic cells. As described herein, oncogenes include genes which encode an oncoprotein and oncogenes which act themselves, such as to inactivate tumor suppressor genes or activate a proto-oncogene. In one embodiment, the exogenous DNA is incorporated into genomic DNA of recipient normal cells, which are propagated to produce tumorigenic
20 variants of the parental cells. That the resulting cells are tumorigenic transformants is shown, for example, by culturing them in appropriate medium (e.g., semi-solid medium) and assessing their ability to grow in an anchorage-independent manner and by introducing them into an appropriate animal model (e.g., immunocompromised mice, such as nude mice) and assessing their ability to
25 establish tumors. Tumorigenic cells grow in an anchorage-independent manner and form tumors in immunocompromised mice. The tumorigenic cells can be introduced into animals at a variety of sites and by a variety of routes of administration (e.g., subcutaneously, intraperitoneally, intramuscularly, orally, nasally, rectally, intravenously, intrathecally, intraosseously, topically). An
30 appropriate route and location of administration can be selected in view of the type of tumorigenic cell used. Tumorigenic human cells produced as described herein are a subject of the invention. Such tumorigenic human cells are genetically engineered,

in the sense that they contain exogenous DNA (DNA not present in the human somatic cell as it was obtained). The tumorigenic human cells are, typically, also isolated, in that they are not in a human body at the time the exogenous DNA is introduced into them. It is possible for the exogenous DNA to be introduced into
5 cells (or into ancestor or parental cells) while they are present in (have not been removed from) a human body, but this is unlikely to be done for the obvious reason of the risk to recipient's health. The term tumorigenic human somatic cells, as used herein, is not intended to encompass naturally-occurring human tumors or tumor cells, either as they occur in an individual or after removal from an individual.

10 A tumorigenic human somatic cell of this invention is one having stably incorporated therein and stably expressing exogenous DNA (DNA obtained from a source other than the recipient cell) which, when expressed in a normal human somatic cell, results in production of a tumorigenic human somatic cell that grows in an anchorage-independent manner and forms tumors in immunocompromised mice
15 into which the tumorigenic human somatic cell or its progeny are introduced. In a specific embodiment, the tumorigenic human somatic cell has exogenous DNA incorporated into genomic DNA of the cell. In a further embodiment, the exogenous DNA comprises DNA that encodes human telomerase catalytic subunit and DNA comprising (encoding) at least one oncogene. Alternatively, the tumorigenic human
20 somatic cell comprises DNA that encodes human telomerase catalytic subunit; DNA comprising (encoding) a first oncogene and DNA comprising (encoding) a second oncogene, wherein the first oncogene and the second oncogene function in (have an effect in/upon) two different biochemical pathways in human somatic cells. The DNA can be, for example, cDNA that encodes human telomerase catalytic subunit,
25 cDNA that comprises (encodes) an oncogene that functions in a first biochemical pathway and cDNA that comprises (encodes) an oncogene that function in a second biochemical pathway, wherein the first and the second biochemical pathways are two different pathways. In a further embodiment, the first biochemical pathway and the second biochemical pathway are signaling pathways and the DNA, such as
30 cDNA, that encodes the oncogene that functions in the first biochemical pathway encodes an oncogene that functions in the same signaling pathway(s) as does the mutant *H-ras* oncogene product and the DNA, such as cDNA, that encodes the

oncogene that functions in the second biochemical pathway encodes an oncogene that functions in the same signaling pathway(s) as does the SV4-D large T antigen-encoded oncoprotein. The functional effects of the two oncogenes on their respective signaling pathways in the normal human somatic cells in which human telomerase catalytic subunit is ectopically expressed result in production of tumorigenic human somatic cells.

In a further embodiment, the DNA that encodes human telomerase catalytic subunit encodes hTERT, such as cDNA encoding hTERT; the DNA that encodes an oncogene that functions in a first signaling pathway is DNA, such as cDNA, that encodes an oncogene that functions in the same signaling pathway as does the *ras* oncogene product; and the DNA that encodes an oncogene that functions in a second signaling pathway is DNA, such as cDNA, that encodes an oncogene that functions in the same signaling pathway as does the SV40 LT-encoded oncoprotein.

Tumorigenic cells of the present invention are useful in a variety of contexts. For example, tumorigenic cells can be used to identify a drug that reduces (totally or partially) proliferation of tumorigenic human somatic cells. In this method, tumorigenic human somatic cells ectopically expressing telomerase and exogenous DNA encoding at least one oncogene (one or more oncogenes) are cultured or propagated *in vitro* in the presence of a drug to be assessed for its ability to reduce proliferation of tumorigenic human somatic cells, under conditions appropriate for the drug to enter the cells. The extent to which proliferation of the tumorigenic human cells occurs in the presence of the drug to be assessed is determined and compared with the extent to which proliferation of the tumorigenic human somatic cells occurs under the same conditions, but in the absence of the drug to be assessed. If proliferation occurs to a lesser extent in the presence of the drug to be assessed than in its absence, the drug to be assessed is a drug which reduces proliferation of tumorigenic human somatic cells. Further assessment of the ability of the drug to reduce proliferation can be carried out by introducing tumorigenic human somatic cells of the present invention into an appropriate animal model, such as an immunocompromised mouse; administering the drug to the animal via an appropriate route (one by which it will reach the site(s) of tumor formation by the introduced cells); and comparing the extent to which tumor formation occurs in the

animal (referred to as the test animal) to the extent to which tumor formation occurs in a control animal (an animal of the same type, such as an immunocompromised mouse, which is handled in the same manner as the test animal except that it does not receive the drug which is being further assessed). The drug to be further
5 assessed can be administered to the test animal at the time of introduction of the tumorigenic cells or after the tumorigenic cells have been administered. As a result, it is possible to assess, for example, the ability of the drug to prevent proliferation of the tumorigenic cells (as evidenced by the failure of a tumor to form) or its ability to reduce proliferation (as evidenced by the formation of a smaller tumor in the test
10 animal than in the control animal). In one embodiment of this method, at least one of the DNAs that encode an oncogene product is a DNA characteristic of (present in and preferably known to be required for formation of) human tumor cells. As a result, a drug effective in reducing proliferation of malignant human tumors, can be identified.

15 In a variant of this method, a variety of human tumorigenic cells can be derived from a common normal antecedent cell type (e.g. a mammary epithelial cell); each variety contains a different ensemble or combination of exogenous DNAs, including a telomerase-encoding gene and oncogene(s), each of which has been shown to result in transformation of the normal antecedent cell into a
20 tumorigenic cell. A drug of interest is then tested on these varieties of tumorigenic human cells, in order to ascertain how the specific identities of the genes contributing to the tumorigenic state of each variety influences its responsiveness to the drug in question, thereby revealing how the actions of specific introduced genes affect or influence the responsiveness of a tumorigenic human cell to the drug of
25 interest.

Tumorigenic cells of the present invention are also useful to assess the ability of a drug that inhibits proliferation of such cells to inhibit proliferation of tumorigenic cells to a greater extent than it inhibits proliferation of corresponding normal human somatic cells (human somatic cells of the same type as that from
30 which the tumorigenic cells were produced or derived). That is, they are useful to assess the specificity or selectivity of the inhibitory effect of the drug. Ideally, the drug will inhibit proliferation of only tumorigenic cells or, less desirably, inhibit the

tumorigenic cells preferentially. Drugs that inhibit normal cell proliferation, as well as tumorigenic cell proliferation, are also useful therapeutically. The extent to which this inhibition or proliferation occurs in both types of cells can be assessed by using "pairs" of cells (normal human somatic cells and tumorigenic variants of such human somatic cells produced as described herein) in order to determine the relative responsiveness of the normal human cell type and the tumorigenic variant to drugs under investigation. Such assessment can be carried out *in vitro*, in much the same manner as described above for identifying a drug which reduces proliferation of tumorigenic cells. For example, an *in vitro* method comprises the steps of culturing tumorigenic human somatic cells of the present invention with a drug to be assessed for its ability to inhibit proliferation of tumorigenic human somatic cells to a greater extent than it inhibits proliferation *in vitro* of corresponding normal human somatic cells with the drug; determining the extent to which proliferation of the tumorigenic cells occurs in the presence of the drug and comparing the extent determined with the extent to which proliferation of corresponding normal human somatic cells cultured under the same conditions occurs. If proliferation of the tumorigenic human somatic cells occurs to a lesser extent than does proliferation of corresponding normal human somatic cells, the drug inhibits proliferation of tumorigenic human somatic cells to a greater extent than it inhibits proliferation of corresponding normal human somatic cells.

In a particular embodiment of the present invention, a drug that inhibits the *ras* oncogene product is identified. This method is carried out, for example, by culturing tumorigenic human somatic cells that ectopically express the human telomerase catalytic subunit; DNA encoding a *ras* oncoprotein, such as the mutant H-*ras* oncogene product; and DNA encoding the SV40 LT oncogene product with a drug to be assessed for its ability to inhibit the *ras* oncogene product; determining the extent to which the *ras* oncogene product is present in the cells (referred to as test cells or a first variety of tumorigenic cells) and comparing the extent to which it is present or the extent to which it is active in the test cells with the extent to which it is present or active in control cells. Control cells are the same type of cells as the test cells and are treated in the same manner as the test cells, except that they are cultured in the absence of the drug. In one approach, the ability of the drug to

specifically target the *ras* oncoprotein can be determined by testing such a drug of interest on a second variety of tumorigenic human cells that are derived from the same type of antecedent (parental) normal human cells as are the aforementioned *ras* oncogene-containing tumorigenic cells ("first variety"), but contain and express an ensemble of exogenous DNA (genes) that differs from the ensemble of exogenous genes present in the first variety. That is, the second variety of tumorigenic cell differs from the first variety of tumorigenic cells in that it does not contain and express the *ras* oncogene, but, rather, contains and expresses another (different) oncogene in place of the *ras* oncogene carried by the first variety of tumorigenic cell. The additional exogenous DNA in the second variety is the same as that in the first variety. For example, the first variety of tumorigenic cell can contain and express exogenous DNA encoding human telomerase catalytic subunit, exogenous DNA encoding mutant H-*ras* oncogene product and exogenous DNA encoding SV40 LT antigen oncoprotein. The second variety of tumorigenic cell contains and expresses exogenous DNA encoding human telomerase catalytic subunit, exogenous DNA encoding an oncogene product that does not encode mutant H-*ras* oncogene product or SV40 LT antigen oncoprotein and exogenous DNA encoding SV40 LT antigen oncoprotein. The extent to which the drug inhibits proliferation of the first variety of tumorigenic cells and the second variety of cells is determined. The extent to which the drug inhibits proliferation of the first variety of tumorigenic cells is compared with the extent to which the drug inhibits proliferation of the second variety of cells. If proliferation of the first variety of tumorigenic cells is inhibited and proliferation of the second variety of tumorigenic cells is not inhibited, the drug is a drug which selectively inhibits the H-*ras* oncogene product. In a similar manner, a drug which selectively inhibits another oncoprotein, such as the SV40 LT antigen oncoprotein, can be identified.

In another embodiment, the present invention is a method of identifying a gene or genes whose presence or expression in a tumorigenic human somatic cell produced as described herein is related to or involved in metastasis and/or invasiveness of such cells *in vivo*. In the method, a candidate gene is introduced into tumorigenic cells of the present invention, thereby producing modified tumorigenic human somatic cells; the resulting modified cells are introduced into an appropriate

animal, such as an immunocompromised mouse; the animal is maintained under conditions appropriate for formation of tumors and metastasis to occur and a determination is made of whether metastasis occurred or not. If metastasis occurs, the candidate gene is a gene whose presence or expression in tumorigenic cells is related to or involved in metastasis *in vivo*.

In a similar manner, a gene or genes whose presence or expression in a tumorigenic human somatic cell is related to or involved in invasiveness of the tumor can be identified. In the method, a candidate gene is introduced into tumorigenic human somatic cells of the present invention, thereby producing modified tumorigenic human somatic cells; the resulting modified cells are introduced into an appropriate animal, such as an immunocompromised mouse; the animal is maintained under conditions appropriate for formation of tumors and invasion to occur and a determination is made of whether the tumor invades or not. If invasion occurs, the gene is a gene whose presence or expression in tumorigenic cells is related to or involved in invasion *in vivo*. Identification of a gene or genes related to or involved in metastasis and/or invasiveness of tumorigenic cells can be carried out separately, as described above, or in a combined method in which the metastatic properties and the invasiveness of tumorigenic cells are assessed. Also the subject of this invention are modified tumorigenic human somatic cells. Such cells are tumorigenic human somatic cells additionally altered by introduction of one or more genes, whose expression and/or function in conjunction with the expression and/or function of the exogenous DNA in the tumorigenic human tumor cells causes metastasis and/or invasion to occur. Such modified cells additionally comprise exogenous DNA whose expression and/or function causes metastasis and/or invasion of the cells in an animal (e.g., an immunocompromised mouse) into which they are introduced.

Identification of genes whose expression or function in tumorigenic cells results in metastasis and/or invasiveness of the tumorigenic cells provides a model and a method by which drugs that inhibit (partially or totally) metastasis, invasiveness or both can be identified. For example, modified tumorigenic human cells produced as described herein are introduced into an appropriate animal model, in which metastasis and/or invasion of tissue by the modified cells has previously

been shown to occur. Drugs to be assessed for their inhibitory effects(s) can be administered prior to, simultaneously with or after introduction of the modified cells into the animal. The time at which a drug to be assessed is administered (prior, with or after introduction of cells) makes it possible to determine, for example, if the drug is effective in inhibiting tumor formation and metastasis and/or invasion; inhibiting metastasis and/or invasion but not tumor formation; or reversing tumor formation or metastasis and/or invasion. This aspect of the invention is preferably carried out with modified tumorigenic human somatic cells in which at least one (one or more) of the exogenous DNAs is characteristic of malignant human tumor cells found in individuals with cancer that metastasize and/or invade tissues. Such modified tumorigenic human somatic cells and their use to identify drugs are also the subject of this invention.

It is also possible, as a result of the work described herein, to assess the tumor-specific products (e.g., RNA, proteins) displayed by the tumorigenic human somatic cells and compare them with the products displayed by the corresponding normal human somatic cell and, thus, determine differences (enhanced or lesser display of the products) between the tumorigenic and the normal parental cells. The tumor-associated products include the products of exogenous DNA(s), as well as the products of endogenous genes, active in the tumorigenic cells, including the products of genes present in the genome of the parental normal cell prior to introduction of the telomerase- and oncogene-encoding genes, as described herein. This provides the basis for a method of distinguishing analytically between tumorigenic and normal cells. The results of such differences are useful to identify gene products (RNA or protein) that can be used to detect the presence of tumor cells in the human body, thus making it possible to diagnose the presence of tumor cells in an individual in whom a tumor has previously not been diagnosed or in an individual previously diagnosed as carrying tumorigenic cells (e.g., a tumor) and being treated (e.g., surgically and/or with radiation and/or chemotherapy), in order to assess the individual's response to the treatment(s). Thus, this provides a method for detecting the presence and/or the number of tumorigenic cells; this ability to detect the presence and/or the extent to which tumor cells are present in an individual is useful for diagnostic purposes, as well as for monitoring an individual's

status (the extent to which tumorigenic cells are present in the individual), such as after surgery or during chemotherapy or radiation therapy. In the method of detecting the presence and/or extent to which tumorigenic cells are present in an individual, a biological sample (e.g., a tissue or fluid sample or sample of cells) is obtained from an individual to be assessed for such cells (e.g., an individual to be diagnosed for the presence (or absence) and/or extent of the presence of such cells or an individual who is undergoing therapy and being monitored for response to the treatment). If necessary, the sample is treated in a manner such that cells are rendered available for analysis of the presence and/or levels of tumor-specific products. If the sample is a tissue sample, it is treated, if necessary, in such a manner that cells in the sample can be assessed, using a reagent, such as antibodies or nucleic acids, for the presence and/or levels of tumor products which are not present in normal (non-tumor) cells or are present at a different (higher or lower) level in tumor cells than in normal cells. Cells obtained from the individual may also be treated, if necessary, in such a manner that tumor-specific products can be assessed. The resulting sample, whether treated or not, is referred to as a biological sample to be analyzed. The biological sample to be analyzed is combined with a reagent to be used in the assessment, under conditions appropriate for the reagent to bind or otherwise interact with tumor product(s), which are products displayed by or present in tumorigenic cells, but not displayed by or present in normal cells of the same type and/or products displayed by or present in tumorigenic cells at a level different from (higher or lower) the level at which they are displayed by or present in normal cells (e.g., kidney cells are being assessed for the presence and/or extent or tumorigenic cells and normal kidney cells are used for comparison as a control). The presence or absence of the tumor product(s) and/or the level to which they are displayed by or present in tumorigenic cells is determined. Results of the analysis can be compared with results of the same analysis carried out on normal cells, if needed. If tumor products are shown to be present in the biological sample and/or are shown to be present at different levels in the biological sample than in normal cells, this is indicative of the presence of tumor products and, thus, is indicative of the presence of tumor cells in the biological sample. Presence or absence can be determined or the extent to which tumor cells are present in the biological sample

can be assessed, using known methods. The extent to which tumor cells are present in the biological sample can be particularly valuable information to have available in the instance in which the response of an individual to therapy is being assessed or monitored.

5 Detection can be carried out, for example, using antibodies that specifically recognize protein products displayed by or present in tumorigenic cells, but not displayed by or present in normal cells or antibodies that recognize proteins that are expressed at different levels (higher or lower) in the tumorigenic cells than in the normal cell. The identity(ies) of such products are determined by comparison of the
10 relative expression patterns of normal human cells and their derived tumorigenic variants, as described herein. For example, the expression of a particular protein may be found to be specific to a tumorigenic human kidney cell (and not to occur in the normal human kidney cell). Alternatively, expression of the protein may be found to be higher in the tumorigenic kidney cell than in the antecedent normal
15 human kidney cells. Analytical methods, including use of antibodies, can be used as diagnostic tools to determine the levels of such proteins in the body of an individual (e.g., in the body fluids, the kidneys, or in distant locations within the body of such individual) as a means of detecting the presence of kidney cancer cells in the individual or gauging the number of such cells present in the individual. These
20 analyses may be performed in order to detect the presence of such a kidney cancer in the body of an individual who has previously not been diagnosed with cancer, or in the body of an individual with already-diagnosed kidney cancer in order to ascertain the stage of progression of the diagnosed tumor. In addition, this approach can be used to determine the presence of and/or the extent to which tumorigenic cells are
25 present in an individual, previously diagnosed with such a tumor, following treatment (e.g., by drug, radiation and/or surgical treatment). Alternatively, nucleic acids can be used as the reagent.

 The present invention provides a method for identifying genes that are expressed in tumorigenic human somatic cells but not in the corresponding
30 (antecedent or parental) normal human somatic cell or are expressed at different levels in the two types of cells. In the method, a tumorigenic human somatic cell (a variant of a normal human somatic cell) ectopically expressing a telomerase

catalytic subunit (e.g., human telomerase catalytic subunit) and at least one oncogene and the normal (parental) human somatic cell of which the tumorigenic cell is a variant are assessed for the presence of products, to determine if a gene product(s) is expressed in the tumorigenic cell, but not in the corresponding normal cell, or is expressed at a different (higher or lower) level in the tumorigenic cell than in the parental normal cell. The present invention makes available "pairs" of cells (a normal human somatic cell and a tumorigenic variant thereof) useful for identifying gene products that are specifically expressed, up-regulated or down-regulated in tumor cells, compared with the normal parental cells. For example, a tumorigenic human somatic cell ectopically expressing human telomerase catalytic subunit and one, two or more oncogenes (e.g., a mutant *H-ras* oncogene and SV40 large T antigen oncoprotein) and a cell of the same type as the normal parental human somatic cell from which the tumorigenic variant is produced are assessed for differences in gene products. The cells are analyzed to identify gene products expressed in one cell type (tumorigenic or normal) but not in the other cell type and gene products expressed at different levels (e.g., higher or lower in tumorigenic cells than in normal cells) in the two types of cells. As a result, gene products whose presence, absence and/or level of expression make it possible to distinguish tumorigenic from normal cells are identified. Such gene products can be proteins or nucleic acids (RNA, DNA) and can be detected using known methods, such as through the use of antibody reagents or nucleic acid reagents, which can be made using known methods. The gene products identified in this manner can be used to detect corresponding gene products (and, thus, tumor cells) in samples (tissues, fluids) obtained from individuals (e.g., humans) to be assessed for the presence/absence, extent or occurrence of tumor cells. This provides a method of assessment of cells not available until the present invention.

The present invention also relates to reagents useful to detect and/or quantify tumorigenic cells. The reagents can be, for example, antibodies which recognize products displayed by or present in tumorigenic cells but not displayed by or present in normal cells; they can also be nucleic acids which specifically recognize tumorigenic cell products. Such reagents can be produced using methods known to those of skill in the art. The antibodies can be monoclonal or polyclonal.

The present invention is illustrated by the following examples, which are not intended to be limiting in any way.

Methods

The following methods were used in the work described herein.

- 5 Generation of cell lines. To create amphotropic retroviruses, Phoenix cells were transfected with pBABE-hygro-hTERT (Counter, C. M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:14723-14728 (1998)) and pBABE-puro-*ras*-V12 (gift of S. Lowe) by calcium-phosphate precipitation. These ecotropic retroviral supernatants and supernatants collected from the LT retroviral producer line (ψ :SV40 (Jat, P. S., *et al.*,
10 *Mol. Cell. Biol.* 6:1204-1217 (1986)) were used to infect the amphotropic packaging cell line PT67 (Clontech Laboratories, Palo Alto, CA). The resulting supernatants were used to infect HEK (HA1) (Counter, C. M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:14723-14728 (1998)) cells or foreskin fibroblasts (BJ). Retroviral constructs were introduced serially with drug selection used to purify cell populations between
15 infections. Cells were selected in hygromycin (100 g ml^{-1} , 7 days), puromycin (0.5 g ml^{-1} , 2-3 days), or neomycin (400 g ml^{-1} for 7 days), respectively. Retroviral vectors carrying only drug resistance genes were used as controls. In all cases, the point when a culture reached confluence in a 10-cm culture dish after the last viral infection was designated pd 0; this point represents at least 70 pd from the original
20 primary culture. Cells were considered to have entered crisis when they could no longer be passaged and exhibited widespread cell death.

- Telomerase assays, RT-PCR, telomere analysis, and immunoblotting. Cellular extracts were assayed for telomerase activity using a PCR-based TRAP assay (Kim, N. W. and Wu, F., *Nucleic Acids Res.*, 25:2595-2597 (1997)). For RT-
25 PCR, total cellular RNA was prepared from cells using RNazol (Tel Test B, Friendswood, TX). In each reaction, 100 ng of total RNA was used with primers specific for retrovirally encoded *hTERT* (5' GACACACATTCCACAGGTCG 3'; SEQ ID NO.:1 and 5' GACTCGACACCGTGTACCTAC 3' SEQ ID NO.: 2) or primers specific for human GADPH (5' GAGAGACCCTCACTGCTG 3'; SEQ ID
30 NO.: 3 and 5' GATGGTACATGACAAGGTGC 3'; SEQ ID NO.: 4) using the RTth kit (Perkin Elmer, Foster City, CA). The reverse transcriptase reaction was

performed for 10 min at 70°C followed by thirty cycles of PCR (94°C, 45 s; 60°C, 45 s; 72°C, 90 s). Telomere length was measured by hybridizing a ³²P-labeled telomeric (CCCTAA)₃ (SEQ ID NO.: 5) probe to *Hinf*I- and *Rsa*I-digested genomic DNA. Immunoblotting of total cellular extracts (75 (g) was performed using the rat
5 anti-Ras antibody 259 and the mouse anti-LT antibody Pab 101 (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). Analysis of retroviral integration sites. Genomic DNA (15 (g) from parental and explanted tumor cells was digested with *Eco*RI and *Bam*HI and hybridized to a ³²P-labeled *Eco*RV-Sal I fragment of the *hTERT* cDNA under conditions that did not permit detection of genomic fragments
10 of *hTERT*.

Soft Agar Assays. Soft agar assays were performed as described by Cifone and Fidler. (Cifone, M. A. and Fidler, I. J., *Proc. Natl. Acad. Sci. U.S.A.*, 77:1039-1043 (1980)). Cultures were coded and scored in a blinded fashion by a second observer.

15 Tumorigenicity assays. Immunodeficient mice (Balb/c-ByJ-Hfh11^{nu}, Jackson Laboratory, Bar Harbor, ME) were maintained in pathogen-free conditions. Mice were irradiated with 400 rad prior to injection to suppress natural killer cells (Feuer, G. *et al.*, *J. Virol.*, 69:1328-1333 (1995)); tumors also formed in mice that had not been irradiated but had a 1-2 week latency period. Cells (2 X 10⁶) were
20 injected subcutaneously into mice anesthetized with Metofane, and tumors were measured every 2-3 days. Mice were sacrificed when tumors exceeded 1 cm. In experiments where cells were re-isolated, tumors were removed surgically, minced, incubated in a dilute (0.15%) solution of collagenase for 2 h, washed, and placed in culture. Tumor volume was calculated using the formula $\frac{4}{3} r^3$, where r is the radius
25 of the tumor.

Example 1. Determination of Effect of Ectopic Expression of hTERT in Human Cells

To determine whether human cells immortalized by the ectopic expression of *hTERT* were tumorigenic, combinations of *hTERT* and an oncogenic *ras* (H-*ras*-V12) allele (Serrano, M., *et al.*, *Cell*, 88:593-602 (1997)) were serially introduced
30 using amphotropic retroviruses into human embryonic kidney (HEK) cells that

express LT to bypass senescence (HA1 cells) (Counter, C. M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:14723-14728 (1998)). LT and combinations of *hTERT* and oncogenic *ras* were also introduced into early passage (passage 5), normal human BJ fibroblasts. For each infection, parallel cultures were infected with control
5 retroviruses specifying only a drug resistance gene as controls. Polyclonal, mass-infected populations as well as clonal isolates were obtained for each combination of serially introduced genes. Approximately 60 population doublings (pd) were expended during the process of serially introducing LT, *hTERT*, and *ras*. Of note, cells expressing only *hTERT* and *ras* could not be propagated, as these cells entered
10 a senescent state immediately after the introduction of *ras*, (Serrano, M., *et al.*, *Cell*, 88:593-602 (1997)), confirming that *hTERT* does not abrogate a *ras*-induced growth arrest (Morales, C. P. *et al.*, *Nature Genetics*, 21:115-118 (1999)). Expression levels of LT were similar in all cell populations and cell clones and increased expression of the Ras protein was observed in the expected cell populations and clones following
15 infection with a vector transducing the *ras* oncogene. The ectopic expression of *hTERT* resulted in telomerase activity in both HEK and BJ cells. This telomerase activity resulted in both telomere elongation and stabilization in these cells as assessed by Southern blotting of genomic DNA.

Expression of the *ras* oncogene did not affect the ability of telomerase to maintain
20 telomeres in these cells.

Expression of *hTERT* led to immortalization of BJ fibroblasts expressing LT, recapitulating the results seen previously with HEK and pre-senescent BJ cells (Figures 1A and 1B). Bodnar, A. G. *et al.*, *Science*, 279:349-352 (1998); (Counter, C. M. *et al.*, *Proc. Natl. Acad. Sci. USA*, 95:14723-14728 (1998)). In contrast,
25 telomerase-negative polyclonal (Figures 1A and 1B) and monoclonal cell populations expressing only LT and *ras* entered crisis within 10 pd, and no immortal clones arose spontaneously from these cultures after two months of culture, confirming earlier observations (O'Brien, W., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 83:8659-8663 (1986)). Oncogenic *ras* led to clear morphological transformation,
30 but had only a minor effect on the growth rate in monolayers of BJ fibroblasts expressing both LT and *hTERT*.

Example 2. Assessment of Ability of Cells to Grow in an Anchorage-Independent Fashion

The ability of these various cell types to grow in an anchorage-independent fashion, one of the hallmarks of the tumorigenic state, was then assessed. Efficient colony formation in soft agar was observed only with cells expressing the combination of LT, *ras* and *hTERT* (Table 1). Although occasional colonies were seen with cells expressing only LT and *ras*, these were both significantly less numerous, as well as dramatically smaller in size. Furthermore, when cells expressing only LT, LT and *ras*, or LT and *hTERT* were introduced into immunodeficient nude mice, no tumors were observed even after three months of observation, although the telomerase-expressing human breast cancer cell lines BT549 or SW613, used as controls, readily formed tumors in this assay (Table 2).

In marked contrast, when cells expressing LT, *ras* and *hTERT* were introduced into nude mice, rapidly growing tumors were repeatedly observed with high efficiency (Table 2, Figure 2B). Histologically, these converted HEK cells formed malignant tumor nodules composed of cells with cytoplasmic vacuoles, while the BJ fibroblasts were transformed into large, malignant, undifferentiated, spindle to epithelioid-shaped cells. Thus, ectopic expression of these three genetic elements appeared to be sufficient to confer tumorigenic potential on both HEK cells and human fibroblasts.

It remained possible, however, that additional genetic alterations were required beyond these three changes in order for these cells to become tumorigenic. Several observations made such a scenario unlikely. No lag was observed in the outgrowth of tumors following injection of these cells into mice (Figure 2B). Analysis of retroviral integration sites by Southern blotting revealed that the cell populations expressing LT, *hTERT*, and *ras* were polyclonal both before injection into and after recovery from nude mice. Explanted tumor cells were morphologically indistinguishable, had similar telomere lengths, grew *in vitro* at the same rate (Figure 2A), and formed similar numbers of anchorage-independent colonies as the parental cells (Table 1). Furthermore, when these explanted tumor cells were re-inoculated into nude mice, they formed tumors with similar kinetics to those seen following injection of the parental cells (Figure 2B). Taken together,

these findings suggest the tumorigenic growth exhibited by these cells is not the consequence of additional, rare stochastic events occurring *in vivo* following inoculation of these cells.

Thus, data presented herein show ectopic expression of a defined set of
5 genes, specifically LT, *ras* and *hTERT*, suffices to convert normal human cells into tumorigenic cells. These results provide the basis for defining the biochemical pathways that must be disrupted in order to create tumorigenic human cells from normal mesenchymal or epithelial precursors.

Table 1. Anchorage-independent growth of LT-expressing HEK and BJ cells

Experiment 1 ^a											
	HEK + LT				BJ + LT				Calu-1		
	hTERT- Ras-	hTERT- Ras+	hTERT+ Ras-	hTERT+ Ras+	hTERT- Ras-	hTERT- Ras+	hTERT+ Ras-	hTERT+ Ras+			
10 ⁴	10	40	4	160	0	3	0	145	200		
10 ³	0	12	0	80	0	0	0	40	75		
Experiment 2 ^b											
	HEK				BJ				293		
	LT + hTERT + Ras				LT + hTERT + Ras						
	Parental	Explanted			Parental	Explanted					
10 ⁵	3460	2850			4046	4858			2220		
10 ⁴	785	434			524	492			286		
10 ³	106	34			50	64			29		

Numbers on the left indicate the numbers of cells seeded into soft agar. The numbers in under each cell population indicate the number of colonies counted after 21 days. The lung cancer cell line Calu-1 and the adenovirus-transformed cell line 293 were used as positive controls. ^aSix clonal isolates from HEK or BJ cells expressing only LT and hTERT never formed colonies, while clonal isolates from HEK or BJ cells expressing LT, hTERT, and *ras* produced variable numbers of colonies (HEK 0-40 and BJ 7-247; 10⁴ cells seeded). The number of colonies formed by such cells correlated with the expression of *ras* in each clonal isolate (data not shown). ^bParental and explanted refer to cell populations before and after passage in a nude mouse.

Table 2. Formation of tumors in immunodeficient nude mice.

HEK + LT		number of tumors/number of injections
5	hTERT -, Ras -	0/6
	hTERT -, Ras +	0/6
	hTERT +, Ras -	0/8
	hTERT +, Ras +	8/8
BJ + LT		
10	hTERT -, Ras -	0/4
	hTERT -, Ras +	0/4
	hTERT +, Ras -	0/4
	hTERT +, Ras +	4/4
BT549		8/8
SW613		8/8

Results are shown for polyclonal populations. Analysis of 2 individual clones
 15 derived from each population gave identical results. 2×10^6 cells were injected in
 each case.

While this invention has been particularly shown and described with
 references to preferred embodiments thereof, it will be understood by those skilled
 in the art that various changes in form and details may be made therein without
 20 departing from the scope of the invention encompassed by the appended claims.

CLAIMS

What is claimed is:

1. A tumorigenic human somatic cell, the human tumorigenic somatic cell having stably incorporated therein and stably expressing exogenous DNA which, when expressed in a normal human somatic cell, results in production of a tumorigenic human somatic cell that grows in an anchorage-independent manner and forms tumors in immunocompromised mice into which the tumorigenic human somatic cell or its progeny are introduced.
2. The tumorigenic human somatic cell of claim 1, wherein the exogenous DNA is incorporated into genomic DNA of the cell.
3. The tumorigenic human somatic cell of claim 1, wherein the exogenous DNA comprises: (a) DNA that encodes human telomerase catalytic subunit ; (b) DNA that encodes a first oncogene; and (c) DNA which encodes a second, distinct oncogene, wherein the first oncogene and the second oncogene function in two different biochemical pathways in human somatic cells.
4. The tumorigenic human somatic cell of claim 3, wherein the DNA of (a) is cDNA that encodes human telomerase catalytic subunit ; the DNA of (b) is cDNA that encodes an oncogene which functions in a first biochemical pathway; and the DNA of (c) is cDNA that encodes an oncogene which functions in a second biochemical pathway, wherein the first biochemical pathway and the second biochemical pathway are two distinct biochemical pathways.
5. The tumorigenic human somatic cell of claim 4, wherein the first biochemical pathway and the second biochemical pathway are signaling pathways and the cDNA of (b) encodes an oncogene that functions in the

- same signaling pathway as does the mutant H- *ras* oncogene product and the cDNA of (c) functions in the same signaling pathways as does the SV40 large T antigen-encoded oncoprotein, wherein the functional effects of the cDNA of (b) and the functional effects of the cDNA of (c) on their
5 respective signaling pathways in the normal human somatic cell in which human telomerase catalytic subunit is ectopically expressed result in production of a tumorigenic human somatic cell.
6. The tumorigenic human somatic cell of claim 5, wherein the DNA of (b) is cDNA which encodes the mutant H-*ras* oncogene product and the DNA of
10 (c) is cDNA which encodes the SV40 large T antigen oncogene product.
7. The tumorigenic human somatic cell of claim 6, wherein the cell forms tumors in immunocompromised mice into which it is introduced and the tumors formed are non-invasive and non-metastatic in the mice.
- 15 8. Tumorigenic human somatic cells produced by introducing into normal somatic cells exogenous DNA comprising: (a) DNA which encodes human telomerase catalytic subunit; (b) DNA which encodes an oncogene which functions in a first signaling pathway; and (c) DNA which encodes an
20 oncogene which functions in a second signaling pathway, wherein the first and the second signaling pathways are not the same and the DNA of (a), the DNA of (b) and the DNA or (c) are stably incorporated into and stably expressed in the normal somatic cells and progeny thereof.
9. The tumorigenic human somatic cells of claim 8, wherein the exogenous
25 DNA is incorporated into genomic DNA of the cells.
10. The tumorigenic human somatic cells of claim 8, wherein the DNA of (a) is cDNA which encodes *hTERT*; the DNA of (b) is cDNA that encodes an oncogene that functions in the same signaling pathway as does the *ras* oncogene product and the DNA of (c) is cDNA that encodes an oncogene

that functions in the same signaling pathways as does the SV40 LT-encoded oncoprotein, wherein function of the oncogene encoded by the cDNA of (b) and the oncogene encoded by the cDNA of (c) in their respective signaling pathways in the normal human somatic cells in which human telomerase catalytic subunit is ectopically expressed results in production of tumorigenic human somatic cells.

11. The tumorigenic human somatic cells of claim 10, wherein the DNA of (b) is cDNA which encodes the *H-ras* oncogene product and the DNA of (c) is cDNA which encodes the SV40 LT oncogene product.
- 10 12. The tumorigenic human somatic cells of claim 11, wherein the cells form tumors in immunocompromised mice into which they are introduced and the tumors formed are non-invasive and non-metastatic in the mice.
13. A method of producing tumorigenic human somatic cells from corresponding normal human somatic cells, comprising introducing into the normal human somatic cells exogenous DNA which, when expressed in the normal human somatic cells, transforms the normal human somatic cells into tumorigenic human somatic cells which grow in an anchorage-independent manner in semi-solid medium and form tumors in immunocompromised mice into which they are introduced.
- 15 14. The method of claim 13, wherein the exogenous DNA comprises: (a) DNA that encodes human telomerase catalytic subunit; (b) DNA that encodes a first oncogene; and (c) DNA that encodes a second oncogene, wherein the first oncogene and the second oncogene function in two distinct biochemical pathways in human somatic cells.
- 20 14. The method of claim 13, wherein the exogenous DNA comprises: (a) DNA that encodes human telomerase catalytic subunit; (b) DNA that encodes a first oncogene; and (c) DNA that encodes a second oncogene, wherein the first oncogene and the second oncogene function in two distinct biochemical pathways in human somatic cells.
- 25 15. The method of claim 14, wherein the DNA of (a) is cDNA which encodes human telomerase catalytic subunit; the DNA of (b) is cDNA that encodes a first oncogene and the DNA of (c) is cDNA that encodes a second oncogene,

wherein the first oncogene and the second oncogene function in two distinct biochemical pathways in human somatic cells.

16. The method of claim 15, wherein the biochemical pathways are signaling pathways and the cDNA of (b) encodes an oncogene which functions in the same signaling pathway as does the *ras* oncogene product and the cDNA of (c) encodes an oncogene which functions in the same signaling pathways as does the SV40 LT antigen oncoprotein, wherein function of the oncogene encoded by the cDNA of (b) and function of the oncogene encoded by the cDNA of (c) in their respective signaling pathways in the normal human somatic cells in which human telomerase catalytic subunit is ectopically expressed results in production of tumorigenic human somatic cells.
17. The method of claim 16, wherein the DNA of (b) is cDNA that encodes the H-*ras* oncogene product and the DNA of (c) is cDNA that encodes the SV40 large T oncogene product.
18. The method of claim 17, wherein the tumorigenic human somatic cells produced from tumors in immunocompromised mice into which they are introduced and the tumors formed are non-invasive and non-metastatic in the mice.
19. A tumorigenic human somatic cell produced by the method of claim 13.
20. A tumorigenic human somatic cell produced by the method of claim 17.
21. The tumorigenic human somatic cell of claim 3, wherein the exogenous DNA additionally comprises DNA that encodes at least one oncogene in addition to the DNA of (b) and the DNA of (c).
22. The tumorigenic human somatic cell of claim 21, wherein each additional oncogene encoded by the exogenous DNA functions in a distinct

biochemical pathway from the biochemical pathways in which other oncogenes encoded by exogenous DNA expressed in the cell function.

23. The tumorigenic human somatic cell of claim 3, wherein the normal human somatic cell is selected from the group consisting of a cell from one of the following: the central nervous system, the peripheral nervous system, the ocular system, connective tissues, the vasculature, the pulmonary system, the muscular system, hepatic tissue, oral tissue, laryngeal tissue, esophageal tissue, gastric tissue, ampullary tissue, biliary tissue, pancreatic tissue, tissue of the small intestine, tissue of the large intestine, rectal tissue, anal tissue, blood forming tissues, tissues of the male reproductive system, tissues of the female reproductive system, genitourinary tissues and skin.
24. An *in vitro* method of identifying a drug which reduces proliferation of tumorigenic human somatic cells, comprising propagating tumorigenic human somatic cells of claim 1 in the presence of a drug to be assessed for its ability to reduce proliferation of tumorigenic human somatic cells, under conditions appropriate for the drug to enter the cells; determining the extent to which proliferation of the tumorigenic human somatic cells occurs in the presence of the drug to be assessed and comparing the extent determined with the extent to which proliferation of the tumorigenic human somatic cells occurs under the same conditions, but in the absence of the drug to be assessed, wherein if proliferation occurs to a lesser extent in the presence of the drug to be assessed than in its absence, the drug to be assessed is a drug which reduces proliferation of tumorigenic human somatic cells.
25. The method of claim 24, wherein the tumorigenic human somatic cells are tumorigenic human somatic cells comprising exogenous DNA which, when expressed in a normal human somatic cell, results in production of a tumorigenic human somatic cell that grows in an anchorage-independent manner and forms tumors in immunocompromised mice into which the tumorigenic human somatic cell or its progeny are introduced, wherein the

exogenous DNA is incorporated into genomic DNA of the cell and is stably expressed.

26. The method of claim 24, wherein the tumorigenic human somatic cells are tumorigenic human somatic cells comprising exogenous DNA which, when expressed in a normal human somatic cell, results in production of a tumorigenic human somatic cell that grows in an anchorage-independent manner and forms tumors in immunocompromised mice into which the tumorigenic human somatic cell or its progeny are introduced, wherein the exogenous DNA comprises: (a) DNA that encodes human telomerase catalytic subunit; (b) DNA that encodes an oncogene which functions in a first biochemical pathway; and (c) DNA that encodes an oncogene which functions in a second biochemical pathway, wherein the first biochemical pathway and the second biochemical pathway are two distinct biochemical pathways.
27. The method of claim 24, wherein the tumorigenic human somatic cells are tumorigenic human somatic cells wherein the exogenous DNA comprises: (a) DNA that encodes human telomerase catalytic subunit; (b) DNA that encodes an oncogene that functions in the same signaling pathway as does the mutant H-*ras* oncogene; and (c) DNA that encodes an oncogene that functions in the same signaling pathway as does the SV40 large T oncoprotein, wherein the functional effects of the DNA of (b) and the functional effects of the DNA of (c) on their respective signaling pathways in the normal human somatic cell in which human telomerase catalytic subunit is ectopically expressed result in production of a tumorigenic human somatic cell.
28. An *in vitro* method of assessing the ability of a drug which inhibits proliferation of tumorigenic human somatic cells to inhibit proliferation of such cells to a greater extent than it inhibits proliferation of parental normal human somatic cells, comprising culturing tumorigenic human somatic cells

- of claim 3 with the drug; determining the extent to which proliferation of the tumorigenic human somatic cells occurs in the presence of the drug and comparing the extent determined with the extent to which proliferation of corresponding normal human somatic cells cultured under the same conditions occurs, wherein if proliferation of tumorigenic human somatic cells occurs to a lesser extent than does proliferation of corresponding normal human somatic cells, the drug inhibits proliferation of tumorigenic human somatic cells to a greater extent than it inhibits proliferation of corresponding normal human somatic cells.
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- 10 29. An *in vivo* method of identifying a drug which reduces the proliferation of tumorigenic human somatic cells, comprising introducing cells of claim 3 into an appropriate animal, in which such cells proliferate and result in formation of a tumor; administering a drug to be assessed for its ability to reduce proliferation of tumorigenic human somatic cells to the animal,
- 15 referred to as a test animal, and determining whether proliferation of tumor cells is less in the test animal than in a control animal, in which introduction of such cells resulted in formation of a tumor and to which the drug was not administered, wherein if proliferation of tumorigenic human somatic cells is less in the test animal than in the control animal, the drug is a drug which
- 20 reduces proliferation of tumorigenic human somatic cells *in vivo*.
30. The method of claim 29, wherein the tumorigenic human somatic cells are selected from the group consisting of a cell from one of the following: the central nervous system, the peripheral nervous system, the ocular system, connective tissues, the vasculature, the pulmonary system, the muscular
- 25 system, hepatic tissue, oral tissue, laryngeal tissue, esophageal tissue, gastric tissue, ampullary tissue, biliary tissue, pancreatic tissue, tissue of the small intestine, tissue of the large intestine, rectal tissue, anal tissue, blood forming tissues, tissues of the male reproductive system, tissues of the female reproductive system, genitourinary tissues and skin.

31. A method of identifying a drug which selectively inhibits the *ras* oncogene product, comprising:
- a) culturing tumorigenic human somatic cells, referred to as a first variety of tumorigenic cells, produced by introducing into genomic DNA of normal somatic cells DNA comprising: (1) cDNA which encodes human telomerase catalytic subunit; (2) cDNA which encodes the H-*ras* oncogene product and (3) cDNA which encodes the SV40 LT oncogene product, thereby transforming the normal somatic cells into tumorigenic human somatic cells, with a drug to be assessed for its ability to inhibit the *ras* oncogene product;
 - b) culturing tumorigenic human somatic cells, referred to as a second variety of tumorigenic cells, produced by introducing into genomic DNA of normal somatic cells DNA comprising : (1) cDNA which encodes human telomerase catalytic subunit; (2) cDNA which encodes an oncogene other than H-*ras* oncogene product or the SV40 large T antigen oncogene product and (3) cDNA which encodes the SV40 large T antigen oncogene product with the drug to be assessed for its ability to inhibit the *ras* oncogene product;
 - c) determining the extent to which the drug inhibits proliferation of the first variety of tumorigenic cells and the second variety of tumorigenic cells;
 - d) comparing the extent to which the drug inhibits proliferation of the first variety of tumorigenic cells with the extent to which the drug inhibits proliferation of the second variety of tumorigenic cells, wherein if proliferation of the first variety of tumorigenic cells is inhibited and proliferation of the second variety of tumorigenic cells is not inhibited, the drug is a drug which selectively inhibits the H-*ras* oncogene product.
32. A method of identifying a gene whose expression in a tumorigenic cell is related to/involved in metastasis of such cells *in vivo*, comprising:

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- a) introducing a candidate gene into tumorigenic human somatic cells of claim 7, thereby producing modified tumorigenic human somatic cells;
- b) introducing the modified tumorigenic human somatic cells into an animal;
- c) maintaining the animal into which the modified tumorigenic human somatic cells were introduced under conditions appropriate for formation of tumors and metastasis to occur; and
- 10 d) determining whether metastasis of the modified tumorigenic human somatic cells occurs, wherein, if metastasis occurs, the candidate gene is a gene whose expression in a tumorigenic cell is related to/involved in metastasis of such cells *in vivo*.
33. A method of identifying a gene whose expression in a tumorigenic cell is related to/involved in invasiveness of such cells *in vivo*, comprising:
- 15 a) introducing a candidate gene into tumorigenic human somatic cells of claim 7, thereby producing modified tumorigenic human somatic cell;
- b) introducing the modified tumorigenic human somatic cells into an animal;
- 20 c) maintaining the animal into which the modified tumorigenic human somatic cells were introduced under conditions appropriate for formation of tumors and invasion of the tumor into tissues of the animal to occur; and
- 25 d) determining whether invasion of the modified tumorigenic human somatic cells occurs, wherein, if invasion occurs, the candidate gene is a gene whose expression in a tumorigenic cell is related to/involved in invasion of such cells *in vivo*.
34. A method of identifying a tumorigenic cell in a biological sample, comprising treating the sample in such a manner that cells in the sample are rendered available for binding with an antibody; thereby producing a treated
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biological sample; contacting the treated biological sample with antibodies that bind tumorigenic cells of claim 3 but do not bind to parental cells from which the tumorigenic cells were produced and determining if binding of the antibodies to cells in the treated biological sample occurs, wherein if binding occurs, a tumorigenic cell is identified.

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35. A method of identifying a gene product which is expressed in tumor cells but not in normal cells of the same type or a gene product which is not expressed in tumor cells but is expressed in normal cells, comprising analyzing tumorigenic human somatic cells expressing exogenous DNA comprising (a)
- 10 DNA encoding human subunit telomerase catalytic; (b) DNA encoding a first oncogene; and (c) DNA encoding a second, distinct oncogene for gene products; analyzing normal parental human somatic cells of which the tumorigenic cells are a variant for gene products and comparing gene products produced by the tumorigenic cells and the normal parental cells,
- 15 whereby a gene product which is expressed in tumorigenic cells but not in normal parental cells or a gene product which is not expressed in tumorigenic cells but is expressed in normal parental cells is identified, thereby identifying a gene product which is expressed in tumor cells but not in normal cells of the same type or a gene product which is not expressed in
- 20 tumorigenic cells but is expressed in normal cells of the same type.
36. Tumorigenic human somatic cells of claim 3, wherein the exogenous DNA of (b), the exogenous DNA of (c) or the exogenous DNA of (b) and (c) is DNA that comprises an oncogene characteristic of malignant human tumor cells that develop in humans.
- 25 37. Modified tumorigenic human somatic cells of claim 3 which additionally comprise exogenous DNA whose expression and/or function causes metastasis and/or invasion of the cells in an animal into which they are introduced.

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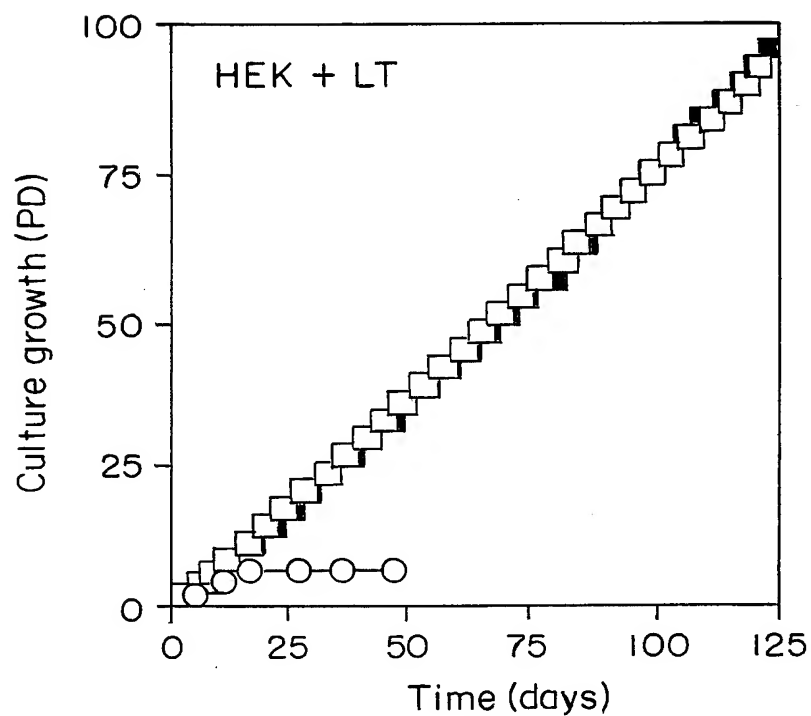


FIG. 1A

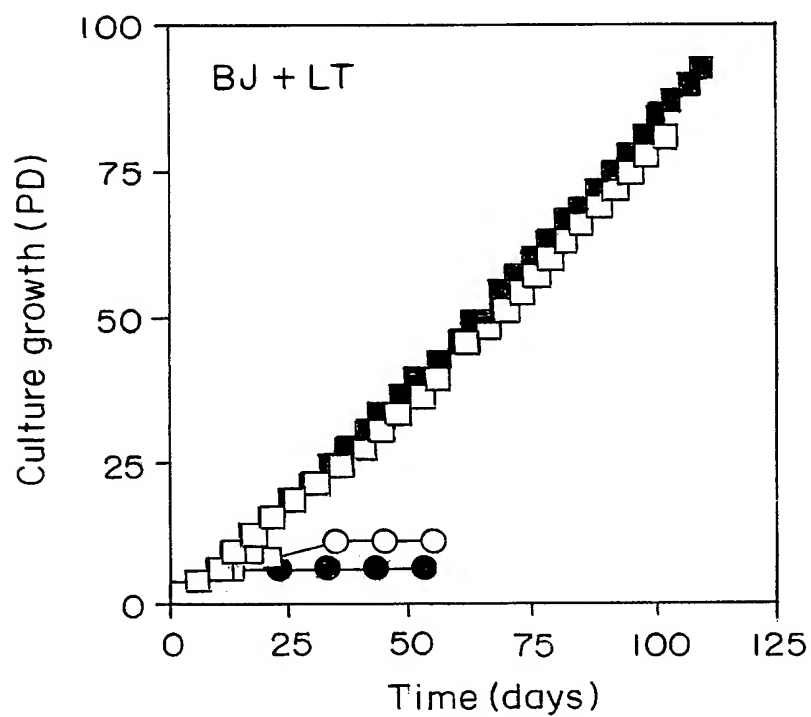


FIG. 1B

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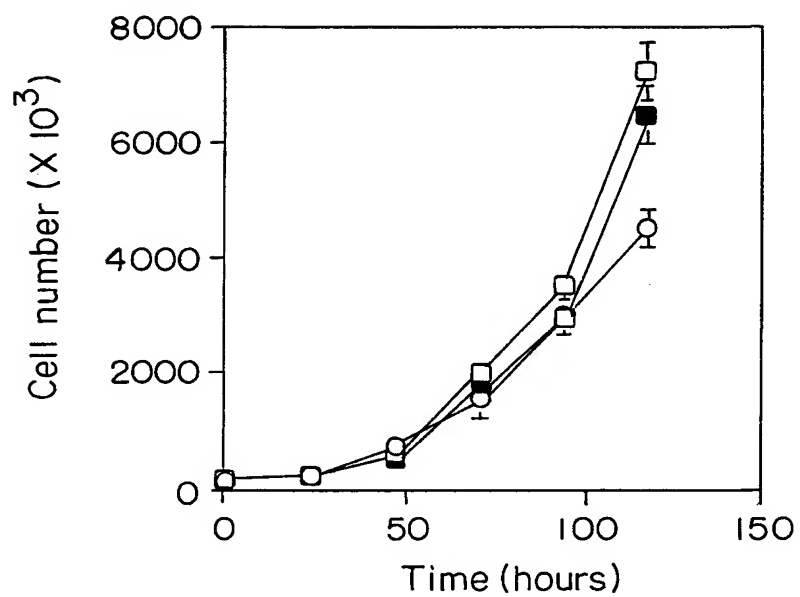


FIG. 2A

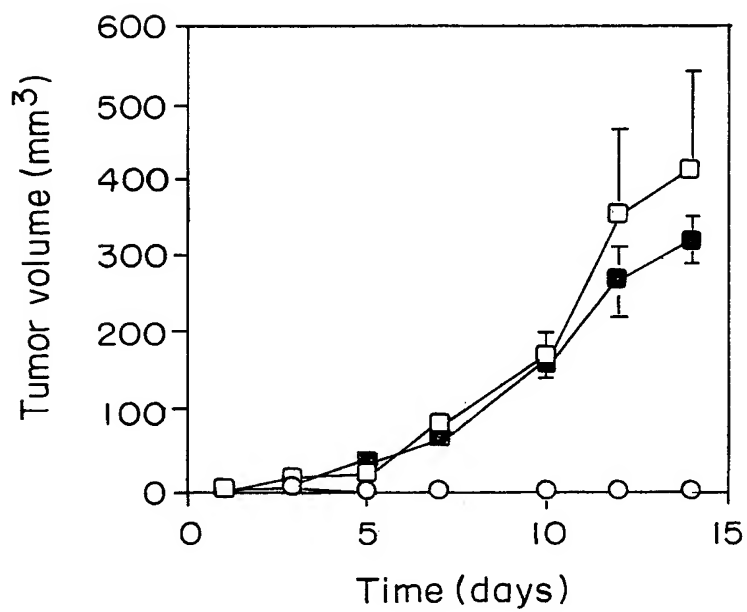


FIG. 2B